

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
(metabolite of Glufosinate Ammonium) to Aquatic Vascular Plants, *Lemna gibba***

EPA MRID Number 48444814

Data Requirement:	EPA DP Barcode	345709
	EPA MRID	48444814
	EPA Guideline	850.4400

Test material: AE C527855 Technical Concentrate (metabolite of Glufosinate Ammonium) **Purity:** 50.9%
w/w

Common name

Chemical name: IUPAC disodium 3-methylphosphinato-propionate

CAS name

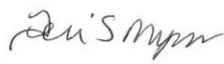
CAS No.

Synonyms disodium salt of AE F061517


Primary Reviewer: Moncie Wright
Staff Scientist, Cambridge Environmental Inc.

Signature: 
Date: 7/26/11

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.

Signature: 
Date: 10/19/11

Primary Reviewer: Catherine Aubee
Biologist, US EPA/OPP/EFED/ERBIV

Signature: 
Date: 12 July 2012

EPA PC Code 128850

Date Evaluation Completed: 12-07-2012

CITATION: Sowig, P. and O. Weller. 2000. Duckweed (*Lemna gibba* G3) Growth Inhibition Test – AE C527855, technical concentrate (Metabolite of Glufosinate-ammonium AE F039866). Unpublished study performed and sponsored by Aventis CropScience GmbH, Frankfurt am Main, Germany. Study completed July 20, 2000.

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EPA MRID Number 48444814

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants duckweed, *Lemna gibba*, were exposed to **AE C527855 Technical Concentrate** (50.9% w/w, disodium salt of MPP metabolite) under static renewal conditions. Nominal concentrations of test substance were 0 (negative control), 20, 36, 64, 112, and 200 mg AE C527855 technical concentrate /L. Nominal concentrations adjusted for purity were 0 (negative control), 10.2, 18.3, 32.6, 57.0, and 101.8 mg MPP (disodium salt)/L. Mean-measured concentrations were <0.69(<LOQ, control), 9.36, 17.1, 31.1, 53.5, and 103 mg MPP (disodium salt)/L. Hereafter, concentrations presented in terms of MPP refer to the MPP disodium salt, unless otherwise specified (as for the analytical reference compound).

There was no toxicity observed in this study, resulting in overall NOAEC and EC₅₀ values of 103 and >103 mg MPP/L, respectively.

There were no compound related phytotoxic effects.

This toxicity study is scientifically sound is classified as **acceptable**. It is consistent with the guideline for a Tier II aquatic plant toxicity study with *Lemna gibba* exposed to a glufosinate transformation product (disodium salt of MPP).

Results Synopsis

Test Organism: *Lemna gibba*

Test Type (Flow-through, Static, Static Renewal): Static renewal

Fronde number, growth rate, and biomass increase

EC₀₅: >103 mg MPP/L 95% C.I.: N/A

EC₅₀: >103 mg MPP/L 95% C.I.: N/A

NOAEC: 103 mg MPP/L

Probit Slope: N/A

Endpoint(s) Effected: None

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EPA MRID Number 48444814

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted according to OECD draft guideline of June 1998: *Lemna*, Growth Inhibition Test; U.S. EPA Pesticide Assessment Guidelines Subdivision J, Hazard Evaluation: Nontarget Plants §123-2: Growth and reproduction of aquatic plants (Tier 2; 1982); U.S. EPA OPPTS 850.4400: Aquatic plant toxicity test using *Lemna* spp., Tiers I and II; and ASTM E 1415-91: Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3 (1991). The study methods and results were assessed according to U.S. EPA OPPTS 850.4400 and OECD Guideline No. 221, and differences and or similarities were described. A deficiency and deviations were noted:

1. The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined. Lack of such information can render a study invalid. However, the control and treatment plants displayed no phytotoxic effects and did not experience significant inhibitions in the endpoints tested.
2. The study author did not report the age of the inocula; OPPTS guidelines suggest that inocula be taken from cultures which are less than 2 weeks old. OECD guidelines suggest that cultures be 7-10 days old.
3. The physico-chemical properties of the test material were not reported.
4. The pre-test health of the plants was not reported.
5. The pH of the control and test solutions ranged from 7.5 to 9.0; OPPTS guidelines suggest that pH be 7.5 ± 0.1 . However, OECD guidance only suggests that pH not drift by more than 1.5 units in the control solutions.

The deficiency and deviations do not have a substantive impact on the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice as adopted by the council of OECD on 26th November, 1997 [C(97)186/(Final)] for implementation at the national level.

A. MATERIALS:

1. Test material	AE C527855 Technical Concentrate (metabolite of Glufosinate Ammonium)
Description:	Clear liquid
Lot No./Batch No. :	Not reported
Purity:	50.9% w/w
Stability of compound under test conditions:	Analytical verification performed on fresh solutions for days 0, 3, and 5 yielded recoveries ranging from 75 to 105% of the nominal test concentrations. The spent solutions from days 3, 5, and 7 had recoveries ranging from 81 to 108% of the nominal test concentrations. The test material was stable under the test conditions.

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
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EPA MRID Number 48444814

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of AE C527855 Technical.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Duckweed, *Lemna gibba* EPA requires a vascular species: *Lemna gibba*.

Strain, if provided: G3

Source: In-house cultures were originally obtained from AgrEvo USA Company, AgrEvo Research Center, Pikeville, North Carolina, which in turn obtained the cultures from the Plant Hormone Laboratory, United States Department of Agriculture, Beltsville, Maryland.

Age of inoculum: 6 weeks

Method of cultivation: Plants were cultured in 20X-AAP nutrient medium.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was not conducted.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	6 weeks	
Culturing media and conditions: (same as test or not)	Same as test (temperature and medium).	
Health: (any mortality observed)	Not reported	

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
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EPA MRID Number 48444814

Parameter	Details	Remarks
		<i>Criteria</i>
<u>Test system</u> Static/static renewal Renewal rate for static renewal	Static renewal Days 3 and 5	 <i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	The test vessels were placed in a waterbath; the incubation facility was not reported.	
Duration of the test	7 days	 <i>EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass 300 mL 150 mL	

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
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EPA MRID Number 48444814

Parameter	Details	Remarks
		Criteria
<u>Details of growth medium name</u> pH at test initiation: pH at test termination: Chelator used: Carbon source:	7.5 8.9-9.0 Yes NaHCO ₃	<hr/> EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Standard medium was used.	
<u>Dilution water</u> source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Deionized water used to create reagent grade water; source not reported 7.5 after adjustment with HCl Filtered by ultrafiltration, ion exchange, and a charcoal unit. Not reported. Not reported. Not reported. Not reported. Not reported.	<hr/> EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.
Indicate how the test material is added to the medium (added directly or used stock solution)	The test substance (0.1 mg) was dissolved in the dilution water to prepare a primary stock solution. Defined amounts of the stock solution were pipetted proportionally into the filled test flasks and mixed by shaking.	

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
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EPA MRID Number 48444814

Parameter	Details	Remarks
		<i>Criteria</i>
Aeration or agitation	Neither	
<u>Sediment used (for rooted aquatic vascular plants)</u> Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	N/A	
<u>Number of replicates</u> Control: Solvent control: Treatments:	3 N/A 3	
Number of plants/replicate	3-5 plants per replicate	
		<i>EPA requires 5 plants.</i>
Number of fronds/plant	2.4-4 fronds per plant	
		<i>EPA requires 3 fronds per plant.</i>
<u>Test concentrations</u> Nominal (not adjusted for purity): Nominal (adjusted for % purity of the test material): Mean-Measured:	0 (negative control), 20, 36, 64, 112, and 200 mg AE C527855 technical concentrate /L 0 (negative control), 10.18, 18.32, 32.58, 57.01, and 101.8 mg MPP/L <LOQ (<0.69, control), 9.36, 17.1, 31.1, 53.5, and 103 mg MPP/L	<i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A- no solvent was used	

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
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EPA MRID Number 48444814

Parameter	Details	Remarks
		Criteria
Method and interval of analytical verification	Samples from the control and all treatment levels were taken from all freshly prepared solutions (days 0, 3, and 5) and all spent solutions (days 3, 5, and 7) and analyzed via HPLC with UV detection (199 nm).	AE F061517 (MPP free acid, 97.9% w/w) was used as a reference compound for analytical verification. Fortification and method validation samples and matrix and solvent blanks were analyzed concurrently. The linearity range for analytical verification was 0.99 to 19.84 mg/L. There is greater uncertainty in measured test concentrations outside this range. Only two of the test concentrations were within this range.
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	24-25°C Continuous Wide spectrum fluorescent lamps of universal white-type L25 58.6-59.9 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	
<u>Reference chemical (if used)</u> name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	- Frond number - Growth rate (based on frond number) - Dry weight increase	
Measurement technique for frond number and other end points	The method of determination for frond number was not reported. Growth rate was determined using logarithmic equation that accounts for the average number	

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
(metabolite of Glufosinate Ammonium) to Aquatic Vascular Plants, *Lemna gibba***

EPA MRID Number 48444814

Parameters	Details	Remarks/Criteria
	of fronds observed at the beginning and end of the test. The increase in biomass was determined by subtracting initial biomass from final biomass.	
Observation intervals	Days 3, 5, and 7	
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes; frond number was 174 fronds/mL at test termination.	
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Frond number averaged 174 fronds/mL in the negative control, yielding reviewer-calculated inhibitions of 1, -1, -1, -1, and -5% in the nominal 20, 36, 64, 112, and 200 mg AE C527855 technical concentrate/L test levels, respectively. The study author did not analyze this endpoint.

Growth rate in the negative control averaged 0.382 days⁻¹, yielding inhibitions of 0, 0, -1, 0, and -2%.

The mean increase in biomass in the negative control was 19.3 mg, yielding inhibitions of -6, 1, -4, -7, and -3%.

No phytotoxic effects were observed.

Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate (metabolite of Glufosinate Ammonium) to Aquatic Vascular Plants, *Lemna gibba*

EPA MRID Number 48444814

Table 3: Effect of AE C527855 Technical Concentrate on frond number of Duckweed, *Lemna gibba*.

Treatment Mean-measured (and nominal) concentrations (mg MPP/L)	Initial frond number/test solution	frond number at			
		3 days	5 days	7 days	
				frond number	% inhibition
Negative control	12	37.3	85.3	174	N/A
9.36 (10.18)	12	35.7	83.3	172	1
17.1 (18.32)	12	37.7	85	175	-1
31.1 (32.58)	12	37.7	81	176	-1
53.5 (57.01)	12	38.3	80.7	176	-1
103 (101.8)	12	40.7	82.7	183	-5
Reference chemical (if used)	N/A				

Table 5: Effect of AE C527855 Technical Concentrate on growth of Duckweed, *Lemna gibba*.

Mean-Measured and (Nominal) Concentrations mg MPP/L	Initial frond number/test solution	Growth rate based on frond number (days ⁻¹ , mean)	Growth rate % Inhibition	Biomass increase (mg)	Biomass % Inhibition
Negative control	12	0.382	N/A	19.3	N/A
9.36 (10.18)	12	0.381	0	20.4	-6
17.1 (18.32)	12	0.382	0	19.2	1
31.1 (32.58)	12	0.384	-1	20.2	-4
53.5 (57.01)	12	0.383	0	20.7	-7
103 (101.8)	12	0.389	-2	19.9	-3
Reference chemical (if used)	N/A				

Table 5: Statistical endpoint values.*

Statistical Endpoint	Frond No.	Growth Rate (based on frond no.)	Biomass Increase
NOAEC or EC ₀₅ (mg technical concentrate/L)	ND	200	200
EC ₅₀ (mg technical concentrate/L)	ND	>200	>200
Reference chemical NOAEC IC ₅₀ /EC ₅₀	N/A		

Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate (metabolite of Glufosinate Ammonium) to Aquatic Vascular Plants, *Lemna gibba*

EPA MRID Number 48444814

* Toxicity values based on nominal concentrations unadjusted for % purity.

B. REPORTED STATISTICS:

The NOEC was determined using ANOVA and General Linear Models with Duncan's Multiple Range Test Procedures using SAS (1989). The nominal concentrations of the technical concentrate were used for analysis.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: There were inhibitions of <5% for frond number, growth rate, and biomass increase. The reviewer visually determined all toxicity values using the mean-measured concentrations.

Frond number, growth rate, and biomass increase

EC₀₅: >103 mg MPP/L 95% C.I.: N/A

EC₅₀: >103 mg MPP/L 95% C.I.: N/A

NOAEC: 103 mg MPP/L

Probit Slope: N/A

D. STUDY DEFICIENCIES:

The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

E. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in complete agreement; there was no toxicity in this study. However, the reviewer included frond number in the analysis and used mean-measured concentrations; therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The experiment was initiated April 30, 1999 and was terminated May 7, 1999.

F. CONCLUSIONS:

This toxicity study is scientifically sound is classified as **acceptable**. It is consistent with the guideline for a Tier II aquatic plant toxicity study with *Lemna gibba* exposed to a glufosinate transformation product (disodium salt of MPP). The most sensitive endpoint could not be determined as there was no toxicity in this study, resulting in overall NOAEC and EC₅₀ values of 103 and >103 mg MPP/L, respectively.

Frond number, growth rate, and biomass increase

EC₀₅: >103 mg MPP/L 95% C.I.: N/A

EC₅₀: >103 mg MPP/L 95% C.I.: N/A

NOAEC: 103 mg MPP/L

Probit Slope: N/A

Endpoint(s) Effected: None

III. REFERENCES:

Organization for Economic Cooperation and Development, Draft OECD Guideline for Testing of Chemicals Guideline: *Lemna*, Growth Inhibition Test, April 1997.

**Data Evaluation Record on the Acute Toxicity of AE C527855 Technical Concentrate
(metabolite of Glufosinate Ammonium) to Aquatic Vascular Plants, *Lemna gibba***

EPA MRID Number 48444814

U.S. Environmental Protection Agency (EPA), 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants; Tier 2 of Non-target Area Testing; §123-2 Growth and reproduction of aquatic plants.

U.S. Environmental Protection Agency (EPA), April 1996, Ecological Effects Test Guidelines: OPPTS 850.4400: Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II; EPA 712-C-96-156; Public Draft.

ASTM (1991). Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3. American Society for Testing and Materials. E 1415-91.

U.S. Environmental Protection Agency (EPA), 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR Part 792). Fed. Reg., Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

SAS Institute Inc., 1989, Release 6.08 TS 407. SAS Institute Inc., Cary, North Carolina 27511.